PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/82, 15/54, 15/53
C12N 15/52, A01H 5/00

A1

(11) International Publication Number: WO 94/11519

(43) International Publication Date: 26 May 1994 (26.05.94)

(21) International Application Number: PCT/GB93/02286

(22) International Filing Date: 5 November 1993 (05.11.93)

(30) Priority data:
9223332.9 6 November 1992 (06.11.92) GB

(71) Applicant *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; Imperial Chemical House, 9 Millbank, London SW1P 3JF (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : FENTEM, Philip, Anthony [GB/GB]; 8a Briarwood, Finchampstead, Wokingham, Berkshire RG11 4XA (GB).

(74) Agent: HUSKISSON, Frank, Mackie; Imperial Chemical Industries plc, Group Patent Services Department, P.O. Box 6, Bessemer Road, Welwyn Garden City, Herts AL7 1HD (GB).

(81) Designated States: AU, BB, BG, BR, BY, CA, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PRODUCTION OF POLYHYDROXYALKANOATE IN PLANTS

(57) Abstract

Genes specifying the biosynthesis of polyhydroxyalkanoates are placed under control of a gene switch and inserted into the genome of an oilseed crop. When the gene switch is induced by the application of its chemical inducer, the plant produces PHA but in the absence of the inducer the crop produces ordinary oilseeds. It is preferred that the inserted genes include targeting sequences which direct expression of the genes to the cytosol or glyoxysome and are expressible during seed germination. If seed of the modified plant is cultivated in the absence of the inducer and harvested it may be utilised as ordinary oilseed or, at the producer's choice, it may be subjected to a malting process in the presence of the switch inducer to produce PHA.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

PRODUCTION OF POLYHYDROXYALKANOATE IN PLANTS

This invention relates to the production of polyhydroxyalkanoate in plants.

Poly-3-hydroxybutyrate is a linear polyester of D(-)-3-hydroxybutyrate. It was first discovered in Bacillus megaterium in 1925. Polyhydroxybutyrate accumulates in intracellular granules of a wide variety of bacteria. The granules appear to be membrane bound and can be stained with Sudan Black dye. The polymer is produced under conditions of nutrient limitation and acts as a reserve of carbon and energy. The molecular weight of the polyhydroxybutyrate varies from around 50,000 to greater than 1,000,000, depending on the micro-organisms involved , the conditions of growth, and the method employed for extraction of the polyhydroxybutyrate. Polyhydroxybutyrate is an ideal carbon reserve as it exists in the cell in a highly reduced state, (it is virtually insoluble), and exerts negligible osmotic pressure.

Polyhydroxybutyrate and related poly-hydroxy-alkanoates, such as poly-3-hydroxyvalerate and poly-3-hydroxyoctanoate, are biodegradable thermo-plastics of considerable commercial importance.

The terms "polyhydroxyalkanoates" and "PHA" as used hereinafter includes polymers of 3-hydroxybutyrate, polymers of related hydroxyalkanoates such as 3-hydroxyvalerate, 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, and also copolymers containing more than one of these hydroxyalkanoates.

Polyhydroxyalkanoate is biodegradable and is broken down rapidly by soil micro-organisms. It is thermoplastic (it melts at 180°C) and can readily be moulded into diverse forms using technology well-established for the other thermo-plastics

materials such as high-density polyethylene which melts at around the same temperature (190°C). The material is ideal for the production of biodegradable packaging which will degrade in landfall sites and sewage farms. The polymer is biocompatible, as well as biodegradable, and is well tolerated by the mammalian, including human, body,its degradation product, 3-hydroxybutyrate, is a normal mammalian metabolite. However, polyhydroxyalkanoate degrades only slowly in the body and its medical uses are limited to those applications where long term degradation is required.

Polyhydroxyalkanoate, produced by the micro-organism Alcaligenes eutrophus, is manufactured, as a copolymer of polyhydroxybutyrate and polyhydroxyvalerate, by Imperial Chemical Industries PLC and sold under the Trade Mark BIOPOL. It is normally supplied in the form of pellets for thermoprocessing. However, polyhydroxyalkanoate is more expensive to manufacture by existing methods than, say, polyethylene. It is, therefore, desirable that new, more economic production of polyhydroxyalkanoate be provided.

An object of the present invention is to provide materials and a method for the efficient production of polyhydroxyalkanoate.

According to the present invention there is provided a plant adapted for the intracellular production of polyhydroxyalkanoate (PHA) comprising a recombinant genome of an oil-producing plant, which genome contains genes encoding enzymes necessary for catalysing the production of PHA together with gene regulatory sequences directing expression of the said genes to target plant cell components, expression of the said genes being controlled by a promoter sequence which is inducible by the application of a chemical inducer.

The genes encoding the enzyme or enzymes necessary

for the catalysis of polyhydroxyalkanoate production may be isolated from a micro-organism such as Alcaligenes eutrophus, Rhodococcus ruber, Pseudomonas oleovorans or Pseudomonas aeruginosa.

The preferred oil-producing plant is selected from the group consisting of oilseed rape, canola, soya and sunflower.

The said enzymes for catalysing PHA production are β-ketothiolase, acetoacetyl-CoA-reductase, polyhydroxy-alkanoate- synthetase and 3-hydroxyalkanoyl CoA dehydratase.

The said gene regulatory sequences preferably direct expression of the polyhydroxyalkanoate genes specifically to the germinating oilseed, for example the germinating embryo.

The said chemically inducible promoter sequence may be the promoter of the gene specifying the 27kd sub-unit of the glutathione-S-transferase enzyme in Zea mays.

Preferably the said gene regulatory sequences direct expression of the polyhydroxyalkanoate genes to the cytosol or to the glyoxysome.

These regulatory sequences include promoter sequences directing expression of the biosynthetic pathway specifically to the germinating seed, and transit peptide sequences targeting the enzymes to appropriate subcellular compartments.

It is preferable, for reasons which will later be explained, that the plant be of a species which produces substantial quantities of oil, rather than starch. Such plant species are well known and are simply referred to as "oil-seed" crops and include, oilseed rape, canola, maize, soya and sunflower. Methods for the genetic transformation of many oil crops are known, for example, methods of transformation using Agrobacterium tumefaciens are suitable for many

but alternative direct DNA delivery methods such as the ballistic method, are also available. Such methods are well-described in the literature and well-known and extensively practised in the art.

The biosynthesis of polyhydroxyalkanoate in Alcaligenes eutrophus from the substance, acetyl-CoA involves three enzyme-catalysed steps, illustrated in Figure 1 herewith.

The three enzymes involved are B-ketothiolase, NADP linked acetoacetyl CoA reductase and polyhydroxybutyrate synthase, the genes for which have gene cloned from Alcaligenes eutrophus [Schubert et al, J. Bacteriol. Vol 170 (1988)]. When cloned into Escherichia coli the three genes are known to facilitate production of polyhydroxyalkanoate up to 30% of the cell weight.

An important preferred feature of this invention is the use of an oilseed plant for expression of the polyhydroxyalkanoate.

A feature of this invention is that expression of polyhydroxyalkanoate (PHA) polymers is obtained in the germinating seed of oilseed crops. Germinating oilseeds produce large amounts of acetyl CoA, the substrate required for PHA synthesis, in the cotyledons as a result of fatty acid oxidation, which occurs during the mobilisation of lipid reserves. PHA would be produced by harvesting the seed of oilseed crop plants transformed with PHA biosynthetic genes from bacterial sources. Then seed would then be germinated in a process akin to malting. During the malting process the seed would produce PHA polymers which can then be extracted.

By virtue of the fact that PHA production is placed under the control of an inducible promoter, the invention provides a "dual-purpose" crop. In the absence of the inducer during plant growth and seed production, the product seed is simply ordinary

oilseed. In the presence of the inducer, the PHA producing genes are switched on and the crop produces PHA. The crop may be harvested as an ordinary oilseed crop and used as such or, should circumstances dictate, the seed may be malted and PHA produced in the malting process.

The acetyl CoA produced by the oilseed during germination is normally destined for respiration, to produce energy and CO_2, or destined for synthesis of sucrose, a process known as gluconeogenesis, via the glyoxylate pathway. The sucrose is then transported to the growing points of the germinating embryo. A feature of this invention is that the acetyl CoA is diverted from this glyoxylate pathway into the production of PHA polymers. There are 4 possible ways of achieving this:

i) Transformation of the oilseed with the genes for the polyhydroxybutyrate (PHB) synthesis pathway from Alcaligenes eutrophus ketothiolase, NADP acetoacetyl CoA reductase and PHB synthase. The gene constructs would gain expression of these enzymes in the germinating seed. Acetyl CoA produced by fatty acid oxidation would then utilised in the production in the production of PHB.

ii) A product of fatty acid oxidation is acetoacetyl CoA, an intermediate of the PHB biosynthetic pathway. It may therefore be possible to gain PHB synthesis in germinating oilseed merely by gaining expression only of the NADP acetoacetyl CoA reductase and PHB synthase genes of Alcaligenes eutrophus, and utilising acetoacetyl CoA produced by the germinating seed.

iii)There is evidence that PHA polymers produced by Rhodococcus ruber contain L(+) isomers of hydroxyalkanoic acid. The PHB synthase of Alcaligenes eutrophus, and specific for D(-) hydroxyalkanoyl CoA substrates. It is possible that the Rhodococcus ruber PHA synthase can utilise L(+) isomers of

hydroxyalkanoyl CoAs as substrates. L(+)-3-hydroxybutyryl CoA is produced as an intermediate of fatty acid oxidation by germinating oilseeds. It is therefore possible that PHA polymers could be produced in germinating oilseeds by gaining expression of the PHA synthase of Rhodococcus ruber, or expression of PHA synthases with similar substrate specificities from other organisms.

iv) L(+)-3-hydroxyalkanoyl CoAs of chain length C_4 to C_{18} are produced as intermediates of fatty acid oxidation in germinating oilseeds. These isomers are not recognised by the PHB synthase of Alcaligenes eutrophus. Species of Pseudomonas oleovorans however produce polyhydroxyalkanoate polymers when fed alkanes or alkanoic acids. The resulting polymers contain hydroxyacid monomers of chain length C6-C12. The monomer substrates for PHA synthesis in these organisms come from fatty acid oxidation. The Pseudomonas oleovorans strains DSM1045, and GH4BH1 produce a mixture of PHB polymer and PHA polymer containing monomers of chain length C6-C12 utilising the products of fatty acid oxidation. The PHA synthase genes of these organisms have been isolated. Expression of these Pseudomonas oleovorans genes in germinating oilseeds therefore could enable these tissues to synthesise PHA polymers directly from the intermediates of fatty acid oxidation. It is possible that polymer synthesis in Pseudomonas oleovorans involves the initial conversion of L(+) hydroxyalkanoyl CoAs to their D(-) isomers via the action of two stereospecific dehydratase enzymes. In this case, expression of such PHA polymers in germinating oilseeds would require expression of these two dehydratases genes in addition to the PHB synthase gene.

For expression in higher plants the bacterial genes require suitable promoter and terminator sequence. For constitutive expression the cauliflower

mosaic virus CaMV35S promoter and nopaline synthase (nos) terminator may be used. It is however preferred to target synthesis of PHA only to the germinating oilseed eg, the germinating embryo of oilseed rape.

A feature of this invention is to target production of PHA specifically to the germinating seed. One way of achieving this is to make gene constructs for all the necessary PHA synthetic genes containing a promoter sequence which directs expression specifically to the seed, and specifically to the period of germination. Such a promoter may be obtained by:

i) Differential hybridisation screening of cDNA libraries obtained from seed at an early stage of germination, with libraries obtained from developing seed, leaf, root and germinating seedlings at a later stage of germination. This approach should yield a gene, and thus a promoter, expressed specifically during the early stage of germination.

ii) Isolation of the promoter sequence of the gene encoding the enzyme lipase. Lipase enzymes are expressed specifically at the early stage of germination. Isolation of the gene requires purification of the protein, obtaining amino acid sequences from peptides derived from the purified protein, using these sequences to reverse clone a cDNA and genomic DNA for lipase by techniques well known to those acquainted with the art.

iii) Isolation of the promoter sequence of a gene for a protease. Serine and cysteine proteases are known to be expressed at an early stage of germination,. Conserved sequence elements have been described for such proteases isolated from a range of organisms. These common sequence elements can be used to amplify a portion of a gene for a protease expressed specifically during seed germination using PCR, with a cDNA library from germinating seed as template, and the common sequence elements as primers. The PCR product

could then be used to isolate a full length cDNA and genomic DNA for the protease, from which the promoter could be isolated.

A second way of targeting PHB production specifically to the germinating seed is to make gene constructs for all the necessary PHA synthetic genes containing a promoter sequence from a gene which is chemically inducible. Such a promoter is that of the glutathione-S-methyl transferase II (GSTII) gene isolated from Zea mays. Expression of this gene is induced by chemicals known as safeners. If PHA biosynthetic genes are placed under the control of this promoter PHA polymers will not normally be produced in oilseed plants tissues. Seed will be produced normally and harvested. A chemical safener compound is then applied to the seed, either as a seed dressing or sprayed onto the seed. The seeds are then germinated ("malted") by incubating in the presence of water, and the germinating seed would then express the PHA biosynthetic genes and produce PHA.

In inserting the polyhydroxyalkanoate genes into eukaryotic cells, consideration has to be given to the most appropriate subcellular compartments in which to locate the enzymes.

Key considerations are the site of production of acetyl CoA, acetoacetyl CoA and 3-hydroxyalkanoyl CoAs, and the available space to store the polymer.

The key pathway producing acetyl CoA in germinating oilseed is fatty acid oxidation. While a small proportion (3%) of fatty oxidation occurs in mitochondria, the major proportion (97%) occurs in the glyoxysome, where the acetyl CoA product is normally used to produce succinate via the "glyoxylate pathway". The succinate produced then enters into the gluconeogenic pathway involving reactions in both mitochondria and cytosol, culminating in the production of sucrose.

While significant storage space for PHA polymers exists in the glyoxysomes, potentially greater storage space exists in the cytosol.

The acetyl CoA generated in the glyoxysome could give rise to acetyl CoA in the cytosol via glyoxysomal citrate synthase, outward transport of citrate to the cytosol, and cleavage of citrate via cytosolic citrate lyase, yielding acetyl CoA. It may be possible to obtain PHB synthesis in germinating oilseed by gaining expression of the three PHB biosynthetic pathway enzymes form Alcaligenes eutrophus in the cell cytosol. If generation of acetyl CoA in the cytosol does not occur at a sufficiently high rate however, it will be necessary to site the Alcaligenes eutrophus ketothiolase enzyme in the glyoxysome to compete directly with glyoxylate cycle enzymes for the acetyl CoA produced by fatty acid oxidation. If the acetoacetyl CoA product produced by this enzyme can not traverse the glyoxysomal envelope membrane, it will be necessary to target the Alcaligenes NADP acetoacetyl CoA reductase to the glyoxysome as well. If the 3-hydroxybutyryl CoA intermediate can not traverse the glyoxysome envelope membrane, it will be necessary to target PHB synthase to the glyoxysome, and achieve PHB synthesis, inside this organelle. If the acetoacetyl CoA or 3-hydroxybutyryl CoA intermediates can however traverse the glyoxysome envelope membrane, it will be possible to express ketothiolase in the glyoxysome and PHB polymerase (and possibly acetoacetyl CoA reductase) in the cytosol, in order to achieve acetyl CoA utilisation in the glyoxysome and PHB synthesis in the cytosol.

As stated above, it may be possible to utilise the acetoacetyl CoA produced as an intermediate of fatty acid oxidation, for synthesis of PHB directly, without requiring expression of the Alcaligenes ketothiolase gene. Again depending on whether acetoacetyl CoA or

3-hydroxybutyryl CoA can be transported from the glyoxysome to the cytosol, it may be necessary to express the Alcaligenes NADP acetoacetyl CoA reductase and PHB synthase in the glyoxysome or cytosol to achieve maximal PHB synthesis.

L(+)-3-hydroxybutyryl CoA is also generated in the glyoxysome as an intermediate of fatty acid oxidation. In order to gain synthesis of PHB containing L(+)-3-hydroxybutyrate monomer units via expression of a PHB synthase (such as that from Rhodococcus ruber) capable of using these as substrates, it will be necessary to target expression of this enzyme to the cytosol, or the glyoxysome, dependent on whether L(+)-3-hydroxybutyryl CoA can be transported from the glyoxysome to the cytosol.

L(+)-3-hydroxyalkanoyl CoAs of chain length C_6-C_{12} are produced in the glyoxysome as intermediates of fatty acid oxidation.

To achieve sythesis of PHAs containing monomer units of chain length C_6-C_{12} is is necessary to gain expression of stereospecific dehydratase enzymes from Pseudomonas oleovorans, capable of converting L(+)-3-hydroxyalkanoyl CoAs to D(-)-3-hydroxyalkanoyl CoAs, and a polymerase from Pseudomonas oleovorans capable of synthesising polymers from D(-)-3-hydroxyalkanoyl CoAs of chain length C_6-C_{12} could also be expressed in the glyoxysome or cytosol dependent on the abilities of L(+) or D(-) hydroxyalkanoyl CoA intermediates to be exported from the glyoxysome.

To achieve maximal expression in germinating seed it may therefore be necessary to gain expression of one or more PHA biosynthetic enzymes in the glyoxysome. There are two means of achieving this:

i) The best documented peroxisomal targeting sequence is a tripeptide serine-lysine-leucine (SKL) located at the carboxy terminus, or within the carboxy terminus

domain of the protein. Tripeptide sequences containing cysteine or alanine in place of the serine, and arginine or histidine in place of the lysine, have also been shown to be effective in targeting non peroxisomal proteins to the peroxisome. The final amino acid of the tripeptide must be leucine. The approach is therefore to insert one of these tripeptide sequences at the carboxy terminus of the protein coding regions of the PHA biosynthetic genes.

ii) Rat peroxisomal thiolases contain an amino terminal 26 or 36 amino acid sequence which is cleaved from the protein during import into the peroxisome. Deletion analysis has shown the first 11 amino acids of this targeting sequence only to be necessary for import into the peroxisome. The approach therefore is to construct PHA biosynthetic genes containing the amino terminal targeting sequence (11, 26 or 36 amino acids) to gain glyoxysomal expression of the necessary PHA biosynthetic enzymes in germinating oilseeds.

To obtain synthesis of polyhydroxyalkanoate polymer in the germinating seed tissues it may be necessary to obtain plants expressing more than one gene encoding PHA biosynthetic enzymes. There are four strategies for achieving this:

i) Plants are individually transformed with PHA pathway genes. Plants containing individual genes are grown up in the glass house and cross pollinated to obtain hybrid plants containing two pathway genes. This procedure is then repeated to produce hybrid plants containing three or more genes.

ii) Plants are sequentially transformed with plasmids containing the individual pathway genes.

iii) Two or three pathway genes are cotransformed into the same plant by simultaneous infection with Agrobacteria containing the individual genes.

iv) Plants are transformed with plasmids containing two or three pathways genes.

A combination of these techniques may be used to obtain expression of two ore more genes in a single plants. For methods (ii) and (iii) it is advantageous to insert individual genes into vectors containing different selectable marker genes to facilitate selection of plants containing two or more PHA pathway genes. Examples of selectable markers are genes conferring resistances to kanamycin, hygromycin, sulphonamides and Basta.

CLAIMS

1. A plant adapted for the intracellular production of polyhydroxyalkanoate (PHA) comprising a recombinant genome of an oil-producing plant, which genome contains genes encoding enzymes necessary for catalysing the production of PHA together with gene regulatory sequences directing expression of the said genes to target plant cell components, expression of the said genes being controlled by a promoter sequence which is inducible by the application of a chemical inducer.

2. A plant according to claim 1 wherein the genes encoding the enzyme or enzymes necessary for the catalysis of polyhydroxyalkanoate production are isolated from a micro-organism.

3. A plant according to claim 2 wherein the micro-organism is Alcaligenes eutrophus, Rhodococcus ruber, Pseudomonas oleovorans or Pseudomonas aeruginosa.

4. A plant according to claim 1 in which then said oil-producing plant is selected from the group consisting of oilseed rape, canola, soya and sunflower.

5. A plant as claimed in claim 1, in which the said enzymes for catalysing PHA production are β-ketothiolase, acetoacetyl-CoA-reductase,

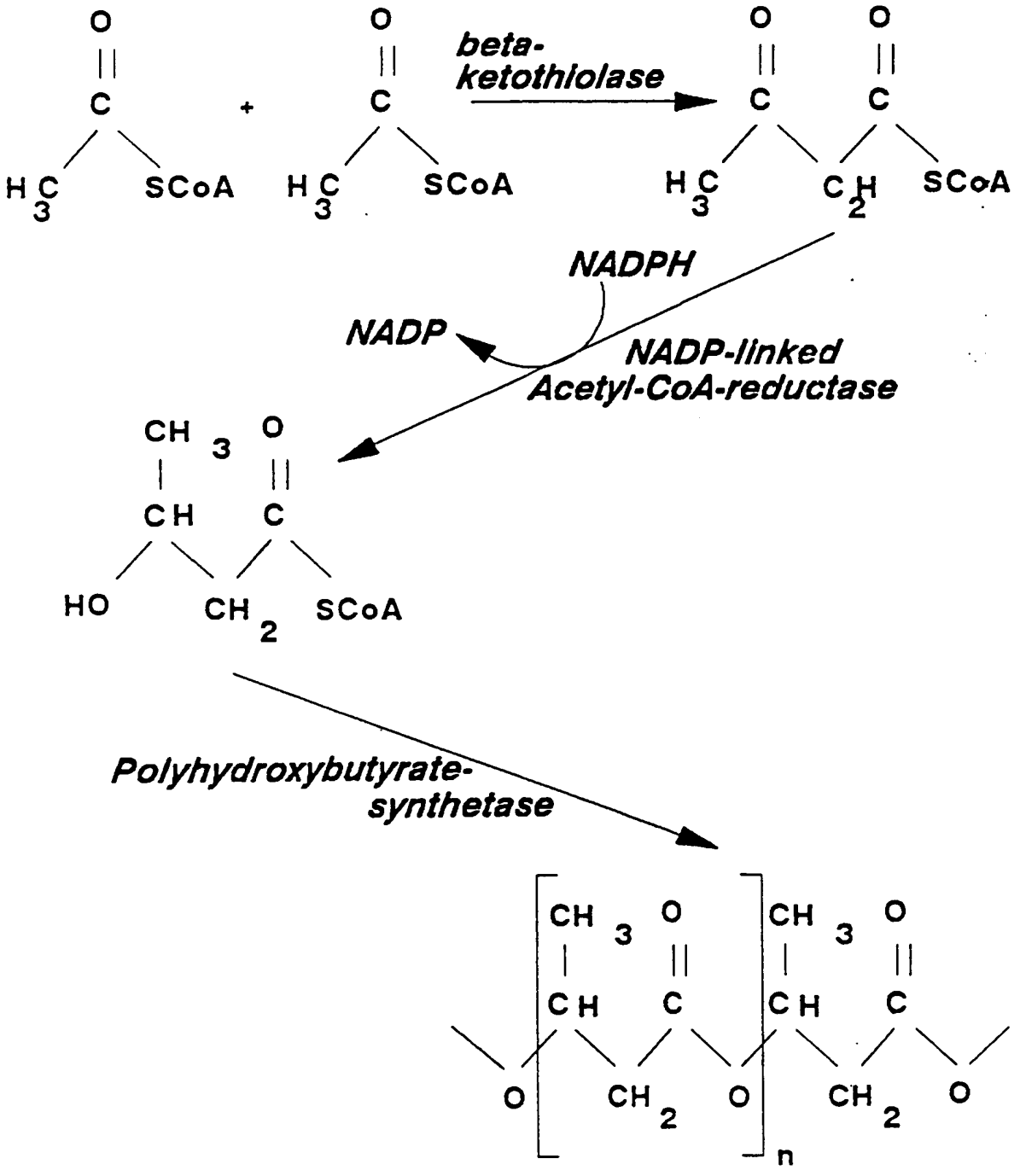
polyhydroxy-alkanoate- synthetase and 3-hydroxyalkanoyl CoA dehydratase.

6. A plant as claimed in claim 1, in which the said gene regulatory sequences direct expression of the polyhydroxyalkanoate genes specifically to the germinating oilseed, for example the germinating embryo.

7. A plant as claimed in claim 1, in which the said chemically inducible promoter sequence is the promoter of the gene specifying the 27kd sub-unit of the glutathione-S-transferase enzyme in Zea mays.

8. A plant as claimed in claim 1, in which the said gene regulatory sequences direct expression of the polyhydroxyalkanoate genes to the cytosol or to the glyoxysome.

FIG. 1


beta-ketothiolase
NADPH
NADP
NADP-linked Acetyl-CoA-reductase
Polyhydroxybutyrate-synthetase

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 93/02286

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/82 C12N15/54 C12N15/53 C12N15/52 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCIENCE vol. 256 , 24 April 1992 , LANCASTER, PA US pages 520 - 523 POIRIER, Y., ET AL. 'Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants' see the whole document ---	1-8
A	WO,A,91 00917 (MIT) 24 January 1991 see the whole document --- -/--	1-8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 February 1994	01.03.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Maddox, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,A	FEMS MICROBIOLOGY REVIEWS.PAPERS PRESENTED AT THE INTERNATIONAL SYMPOSIUM ON BACTERIAL POLYHYDROXYALKANOATES, FRG, 1-5 JUNE 1992 vol. 103, no. 2-4 , 1992 pages 237 - 246 POIRIER, Y., ET AL. 'Perspectives on the production of polyhydroxyalkanoates in plants' see page 244, right column, last paragraph - page 245, left column	1-8
A	SCIENCE vol. 245 , 15 September 1989 , LANCASTER, PA US pages 1187 - 1189 POOL, R. 'In search of the plastic potato' see page 1189, column 2 - column 3	1-9
A	WO,A,90 08826 (ICI) 9 August 1990 see the whole document	7
A	EP,A,0 332 104 (CIBA-GEIGY) 13 September 1989 see the whole document	1-8
A	EP,A,0 388 186 (DU PONT) 19 September 1990 see the whole document	1-8

INTERNAT AL SEARCH REPORT

Information on patent family members

Internat Application No
PCT/GB 93/02286

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9100917	24-01-91	CA-A-	2062816	11-01-91
		EP-A-	0482077	29-04-92
		JP-T-	5500751	18-02-93
		US-A-	5245023	14-09-93
		US-A-	5250430	05-10-93
WO-A-9008826	09-08-90	AU-A-	4947690	24-08-90
		EP-A-	0455667	13-11-91
EP-A-0332104	13-09-89	AU-A-	3108089	14-09-89
		JP-A-	2009377	12-01-90
EP-A-0388186	19-09-90	AU-B-	638448	01-07-93
		AU-A-	5285490	22-10-90
		EP-A-	0463056	02-01-92
		JP-T-	4504054	23-07-92
		WO-A-	9011361	04-10-90